

## THE SEXUAL REPRODUCTIVE DEVELOPMENT OF *PTEROCLADIELLA BULBOSA* (LOOMIS) COMB. NOV. (GELIDIALES, RHODOPHYTA)\*

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**ABSTRACT** — The recently proposed genus *Pterocladia* was based on the sexual development of the type species *P. capillacea*. This study compares similar developments in *P. bulbosa* so as to evaluate morphological variability in the generic characters used.

**RÉSUMÉ** — Le genre *Pterocladia*, récemment proposé, est basé sur le développement sexuel de l'espèce type *P. capillacea*. Cette étude compare des développements similaires chez *P. bulbosa* afin d'évaluer la variabilité morphologique des caractères génériques utilisés.

**KEY WORDS:** Algae, Rhodophyta, *Pterocladia*, *Pterocladia*, *P. bulbosa*, Gelidiales, cystocarp structure.

### INTRODUCTION

The new genus *Pterocladia* was recently proposed (Santelices & Hommersand, 1997) to accommodate those species previously assigned to *Pterocladia* but that have carpogonia directed to both surfaces of the thallus, nutritive filaments growing centripetally forming a virtually solid cylinder around the central axis and cystocarps usually attached to one side of the cystocarp floor, producing chains of carposporangia from the remaining three sides.

All the above features were studied in detail in the type species, *Pterocladia capillacea* (Gmelin) Santelices & Hommersand. Since morphological (Fredriksen & Ruess, 1990; Santelices, 1991a, 1991b, and Guiry & Womersley, 1992) and molecular (Freshwater *et al.*, 1995) evidence suggested three other species should be transferred to *Pterocladia*, Santelices & Hommersand (1997) proposed new nomenclatural combinations for the species previously known under the names of *Pterocladia caerulescens* (Kützinger) Santelices, *P. melanoidea* (Schousboe *ex* Bornet) Dawson and *Gelidiella minima* Guiry & Womersley. However, such transfers did not involve additional morphological

\* It is a pleasure to dedicate this work to Prof. F. Ardré.

studies that could help to evaluate variability in the generic characters used to define *Pterocladia*. Such an evaluation is done here, contrasting the sexual reproductive development of *Pterocladia bulbosa* with *P. capillacea*.

*Pterocladia bulbosa* Loomis is a species restricted to low intertidal and shallow subtidal habitats around the Hawaiian Islands (Loomis, 1960; Santelices, 1977), a pattern of distribution clearly different from *Pterocladia capillacea* that is now known to be widespread in temperate and warm seas. Previous studies (Santelices, 1991b) have recognized both similarities and differences in the cystocarpic architecture of these two species. Therefore, *P. bulbosa* appeared as good material for testing the generic limits of the new genus *Pterocladia*.

### MATERIALS AND METHODS

General procedures followed those described in previous morphological studies of the Gelidiales (Santelices, 1991a, 1991b; Santelices & Flores, 1995; Santelices & Hommersand, 1997). Herbarium specimens as well as specimens fixed and preserved in a 5-10% formalin-seawater solution were used. The representative specimens, and their collection data, used in this study are listed in Table 1. Fertile female gametophytes were examined under a stereomicroscope for cystocarps. A sample of 10 to 20 cystocarps of different sizes, and presumably of different ages, was obtained from each specimen. Cystocarps in herbarium specimens were gradually rehydrated, avoiding tissue damage, and fixed in a 10% formaldehyde solution in seawater. Fixed cystocarps were embedded in

Locality and Date	Determined by	Collection Number
Salt Pond Pavillion, Port Allen, Kauai, Hawaiian Islands M.S. Doty, coll. June 16, 1961	B. Santelices	SS/UC 6797 to 6803
Black Point Beach, Oahu, Hawaiian Isls. P. Ikahara coll. April 27, 1974	B. Santelices	SS/UC 6804
Kappa Island, Oahu, Hawaiian Isls. W. Magruder coll. May 13, 1990	I. A. Abbott	SS/UC 6805 (ex Bish 635290)
Reef flat near Kahului, Maui, Hawaiian Isls. G. Hollenberg coll. April 25, 1965	I. A. Abbott	SS/UC 6806 (ex Bish-502738)

Table 1. Specimens of *P. bulbosa* examined in this study.

gelatin and cut, 30-40  $\mu\text{m}$  thick to avoid destruction of sporogenous tissues, using a Leitz freezing microtome. Transverse, longitudinal and periclinal sections were stained with 1% aniline blue and mounted in 50% Karo<sup>®</sup> corn syrup. Photomicrographs were taken with a Nikon Biophot Microscope. Herbarium specimens are deposited in the algal collection of the Sala de Sistemática, Pontificia Universidad Católica de Chile (SS/UC).

## RESULTS

### External habit

*Pterocladia bulbosa* individuals consist of one to several pectinately branched, erect axes arising from cylindrical creeping axes with small holdfasts at short intervals. Erect axes are cylindrical at the base, up to 0.3 mm in diameter, flattened above, up to 0.7 mm broad and 150  $\mu\text{m}$  thick, ending in acute or rounded tips. Branching is distichous, pectinate or opposite, covering both edges of the flattened axes, starting 1-2 cm above the creeping filaments and rising at short intervals at a broad, almost 90° angle from the margins of the main axes. Most branches are short, 2-4 mm broad, cylindrical, of about 150  $\mu\text{m}$  diameter, slightly clavate, especially if tetrasporangial, and frequently ending in a rounded apex. Intermixed, without any evident order, are larger (up to 3 cm long), flat, and broader branches, similar to the main axes, bearing small, pectinate branchlets (Santelices, 1977).

### Vegetative development

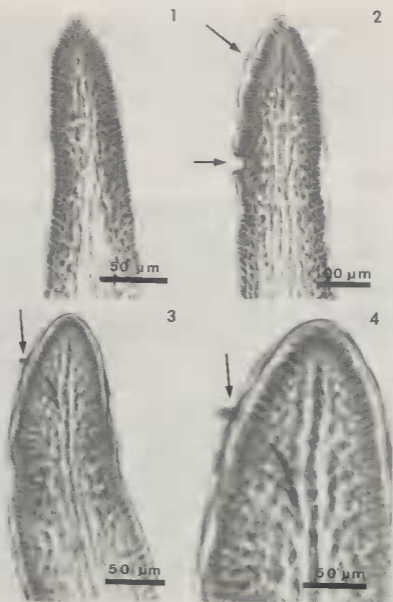
Growth of the thallus of *Pterocladia bulbosa* is initiated by concavo-convex divisions of a dome-shaped apical cell (Fig. 1). Each intercalary cell cuts off two periaxial cells laterally, which function as initials of lateral cell rows of the second order. Each cell in cell rows of the second order divides obliquely cutting off two cells, one toward each surface, which become initials of cell rows of the third order.

Surface cortical cells are generated in groups of four by repeated anticlinal divisions that are perpendicular to one another. Surface cells are quadrangular in shape while still meristematic. They become rounded in middle parts of the frond and elongated in basal parts of axes. In transection the cortex has three layers of cells, the outermost anticlinally elongated, 6 by 12  $\mu\text{m}$ , which grade into sparser, more rounded, internal, cortical cells.

Medullary cells elongate longitudinally, obscuring the central axis a short distance behind the apex. In transection the medullary cells are isodiametric, thick walled and up to 30  $\mu\text{m}$  in diameter. Rhizoidal filaments (rhizines) are easily seen owing to their fine diameters and comparatively thick walls and are moderately abundant around the medullary cells.

### Sexual reproductive development

Fertile female thalli exhibit single cystocarps borne in medial position on short branches. At maturity they are unilocular and inflated on one side, generally with only one ostiole.



Figs 1-4. — Fig. 1. Longitudinal section of a vegetative tip, showing apical cell, axial cells and higher order filaments. Fig. 2. Longitudinal section of an apical tip with ruffled margins (arrows), thickened cuticle and elongated cortical cells, interpreted to be immature spermatangial sori. Figs 3 and 4. Longitudinal section of a fertile female tip showing carpogonium and a large trichogyne (arrows).

Male plants are unknown in this species. In the present study, immature male sori were found close to the tips of cystocarpic branchlets (Fig. 2). In longitudinal sections, these tips exhibit ruffled margins and thickened and raised cuticles. Cortical cells are elongated (5-12  $\mu\text{m}$  long, 3-7  $\mu\text{m}$  in diameter) with a few short longitudinal divisions similar to those exhibited by spermatangial parent cells in other monoecious species of *Gelidium* and *Pterocladia* (e.g. *G. vagum* Okamura, *G. howei* Acleto, *G. pluma* Loomis, *Pterocladia macnabbiana* Dawson and *Pterocladia caerulescens* (Kützing) Santelices & Hommersand).

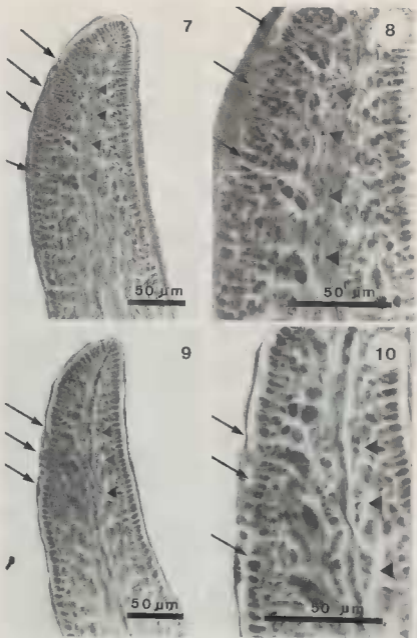
#### Carpogonia, carposporophyte, and cystocarps

Carpogonia are found immediately behind the apex of a fertile branchlet (Figs 3 and 4). They can differentiate at successive distances from the tip on one (Fig. 5) or on both (Fig. 6) frond surfaces. A carpogonium is a terminal or an intercalary cell of the cell row of the third order. Nutritive filaments are borne on basal cells in cell rows of the third order. In longitudinal section, (Figs 7-10) they appear as v-shaped rows, 3-4 cells long, in one or both sides around the axial cell. They can differentiate on both sides of the frond even when carpogonial development occurs only on one side (Figs 9 and 10). In transverse section (Figs 11 and 12) nutritive cells are rounded, nearly isodiametric. They branch forming a ring around each axial segment.

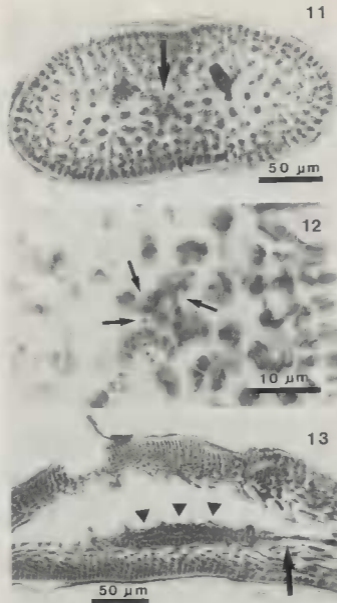
Immature cystocarps (Fig. 13) exhibit a reticulate fusion network that forms a compact tissue around the central axis. The cystocarp wall is pushed up on one side,



Figs 5-6. Longitudinal sections of fertile female pinnules exhibiting carpogonial branches (arrows) to one (Fig. 5) or to both (Fig. 6) frond surfaces.



Figs 7-10. Longitudinal section of fertile female tip showing carpoogonia (arrows) and nutritive filaments (arrow heads). Fig. 7. Carpoogonial cells and nutritive filaments developed on one side of the frond. Fig. 8. Enlargement of Fig. 7. Note V-shaped rows of nutritive cells to one side of the nutritive filaments. Fig. 9. Carpoogonial cells developed on one side of the frond while nutritive cells are on both frond sides. Fig. 10. Enlargement of Fig. 9. Nutritive cells have been indicated only on one side of the frond (arrow heads) but they also occur on the opposite side.

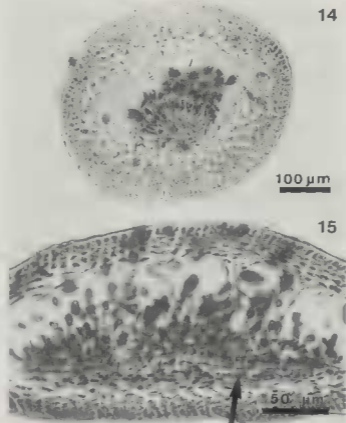


Figs 11-13. — Fig. 11. Cross section through a young, fertile, female pinnule with nutritive cells growing centripetally, forming a ring around the central axis (arrow). Fig. 12. Enlargement of the central part of Fig. 11. Note the rounded, nearly isodiametric shape of nutritive cells (arrows) growing around the central axis. Fig. 13. Longitudinal section through an immature cystocarp. The reticulate fusion network (arrow heads) conforms a compact tissue around the central axis (arrow). The cystocarpic wall is pushed up on one side of the frond.

forming a single cystocarp cavity. Even though the fusion network completely surrounds the central axis, the cystocarp wall is pushed up on only one side of the frond.

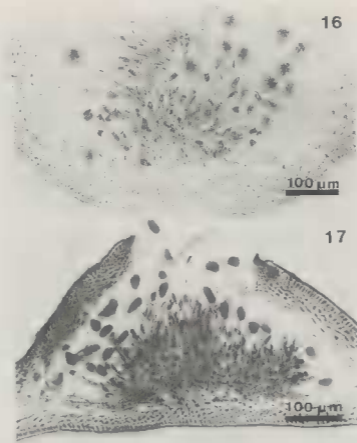
Cystocarps in more advanced stages of development (Figs 14 and 15) exhibit carposporangia in chains at the tip of carposporangial filaments that mature basipetally. The gonimoblast cells on the lower side of the cystocarp link to inner cortical cells and do not form carposporangia. Thus, a transection of the cystocarp at this stage of development shows the gonimoblast attached to the cystocarp floor, producing chains of carposporangia on the remaining three sides (Fig. 14). Carpospores appear as if radiating from a core of filaments surrounding the central axis.

Older cystocarps (Figs 16 and 17) maintain a morphology essentially similar to that described above. The cystocarp remains with only one cavity and the gonimoblasts



Figs 14-15. Fig. 14. Transection of a cystocarp with the carposporophyte developed around the axial cell. The gonimoblast is attached on one side to the cystocarpic floor, producing chains of carposporangia on the remaining sides. Fig. 15. Longitudinal section of a mature cystocarp, producing spores at the apical tips of carposporangial filaments. Axial filaments can still be seen at this stage of development (arrow).





Figs 16-17. Fig. 16. Transection of an old cystocarp. A loose attachment of the gonimoblast to the cystocarp floor can be observed. Fig. 17. Longitudinal section of an old cystocarp. Central axial filaments are no longer visible. Ovoid carpospores are being released through a widely open ostiole.

remain attached to the floor of the cystocarp. Several generations of carpospores are produced which are released through a single ostiole.

## DISCUSSION

While describing the heterogeneity in cystocarpic architecture found in the genus *Pterocladia*, Santelices (1991b) distinguished the *Pterocladia bulbosa*-type of cystocarp from the *P. capillacea*-type. The evidence gathered in this study supports only partially such a distinction, as there are similarities as well as differences between both types of cystocarps.

The most important differences in sexual reproductive development between *Pterocliadiella capillacea* and *Pterocladia bulbosa* occur at early stages of carpogonia and cystocarp development. Thus, while the carpogonia of *P. capillacea* develops on both frond surfaces (Fan, 1961; Santelices, 1991b), those of *P. bulbosa* develop on one or on both surfaces. Nutritive filaments in *P. capillacea* are formed on both sides of the frond, while in *P. bulbosa* they are found on one or on both frond sides. Furthermore, in *P. bulbosa* no strict correlation between bilateral carpogonia production and bilateral differentiation of nutritive filaments exists, as nutritive filaments are found on both frond surfaces even when carpogonia have differentiated on only one frond surface.

Differences appear also in later stages of development. The septum of the cystocarp of *Pterocliadiella capillacea* can appear located close to the middle of the cystocarp. This is due to failure of the gonimoblast to attach properly to the floor of the cystocarp or because of secondary detachment in old cystocarps. Under these circumstances the cystocarp appears as formed by two unequal locules (Fan, 1961; Santelices, 1991a, 1991b). Such a condition was not found in mature stages of cystocarpic development of *P. bulbosa*.

The cystocarpic development of *Pterocladia bulbosa* also shows similarities with the pattern described for *Pterocliadiella capillacea*. Nutritive filaments, irrespective of the differentiation of carpogonial cells on one or two frond sides, are produced on both frond surfaces. They grow centripetally forming a compact tissue around the central axis. Further cystocarpic development is characterized by the linking of the gonimoblast cells on one side of the cystocarp to inner cortical cells on the lower side of the cystocarp, producing chains of carposporangia on the remaining three sides.

When Santelices & Hommersand (1997) described *Pterocliadiella*, they stressed the differences between this new genus, *Pterocladia* and *Gelidium*. The most important difference was the pattern of development of nutritive filaments and the structure of the mature cystocarp. In *Pterocladia*, nutritive filaments are produced only on one side of the frond, that on which the carpogonial branches are formed. In *Gelidium*, the nutritive filaments form a network of short chains around the second order filaments on both sides of the fronds forming a symmetric bilocular cavity with a medial septum. In *Pterocliadiella*, nutritive filaments grow centripetally, forming a virtually solid cylinder around the central axis. Cystocarpic development is asymmetric, with carposporangia production radiating from a core of filaments surrounding the central axis. This important character is also shown by *P. bulbosa*. Therefore, in spite of the differences with *P. capillacea* noted in very early and very late stages of cystocarpic development, *Pterocladia bulbosa* should be transferred to the genus *Pterocliadiella*. The new combination *Pterocliadiella bulbosa* (Loomis) comb. nov. is proposed, based on the basionym *Pterocladia bulbosa* Loomis, 1960, pp. 7-8, pl. 9, figs 2-3; pl. 10, fig. 3; pl. 11, figs 1 and 4.

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